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## INFECTIOUS DISEASES IN 1964

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### New Antibiotics

#### 1. The Semi-Synthetic Penicillins

In the general 1961 review, we reported on the first work done with semi-synthetic penicillins. We might remember that these are obtained by adding varied lateral chains to the level of the radical C-NH<sub>2</sub> of the beta-lactam of 6-amino-penicillinanic acid, the common "nucleus" of all of the penicillins.

Among the many derivatives obtained by this method, only products which exhibit one of the following three qualities have been retained in therapy:

Resistance to penicillinase, an enzyme which is, in particular, secreted by many strains of pathogenic staphylococci.

Resistance to stomach acidity, thus permitting oral utilization.

Spectrum of antibacterial activity expanded to the gram-negative bacilli.

We are going to study these products, focusing only on those which are currently sold in France.

#### (A) Semi-Synthetic Penicillins Which Resist Penicillinase

These act on many strains of staphylococci that resist penicillin G (80% in a hospital environment) while still retaining the latter's advantages: absence of toxicity (with the exception of accidents of intolerance of an allergic nature) and narrow margin between bacteriostatic and bactericidal concentrations. They thus constitute tremendous progress in the

field of anti-infectious therapy.

Methycillin: In 1961, we reviewed the first biological experimentation and the first clinical reports. Since that date, the initial favorable impression has been confirmed by a large number of observations reporting success in the treatment of serious staphylococci involving a strain that resists penicillin G.

A recent publication by Mr Martin emphasizes that it is sometimes useful to employ very strong doses in these extremely serious cases. This involved a septicemia with resistant staphylococci and an abscess of the spleen which was cured by the daily administration of 25 g of methycillin (about 500 mg/kg) up to a total dose of 360 g.

Oxacillin was introduced into therapy more recently.

This is the sodium salt of 5-methyl-3 phenyl-4 isoxazolyl-penicillin. It resists penicillinase and it is moreover stable in an acid medium, which means that it can be used buccally.

All authors who have tested its antibacterial activity have found -- with respect to staphylococci (which secrete penicillinase) -- minimum inhibiting concentrations which are weaker than for methycillin.

On the average, they are less than 1 mug per ml.

For the other germs, the spectrum is the same as that for penicillin G but the effective concentrations are stronger than for the former (particularly for those staphylococci which do not secrete penicillinases).

We must thus reserve oxacillin and methycillin for treatment of staphylococci that resist penicillin G.

The blood rates [counts] obtained orally are weaker and more irregular than those obtained parenterally.

As for the other penicillins, elimination is rapid, principally renal and the sample taking must be repeated every 4 or 6 hours.

Digestive tolerance is good.

The usual oral posology is between 2 and 3 grams every 24 hours in adults and 100 mg/kg and 24 hours in children.

These doses can be exceeded without inconvenience. The diffusion into the LCR [sic] is rather weak. The local ways can be used.

The injectable solutions (IM or IV perfusion) remain stable at least 24 hours after their preparation.

The first series of clinical tests published report highly satisfactory results. For example, Bunn and Amberg obtained therapeutic success in 16 staphylococcias involving a strain resisting penicillin G, Leduc and Fontaine reported success in 20 cases of staphylococcus infections; Rutenburg obtained 40 recoveries in 50 patients revealing staphylococcias due to a strain that secreted penicillinase; Abu-Nassar observed a favorable effect in 29 out of 36 cases. In France, Massias obtained 24 therapeutic successes in a series of 30 surgical staphylococcias which were serious.

However, the remarkable therapeutic successes obtained with methycillin and oxacillin must not cause us to forget the possibility that we might encounter strains of staphylococci that might resist both of these antibiotics.

This resistance certainly develops through a mechanism that differs from the one involved in the secretion of penicillinase. It may be obtained in vitro. It is completely cross-bred between methycillin and oxacillin.

Fortunately, naturally resistant strains, which have been isolated from patients, are rather rare today.

However, Chabbert and Baudens found 10 resistant strains out of 83 tested between February 1961 and February 1962.

The strains tested came from cases which failed to recover, in spite of prior antibiotic therapy, in various hospitals throughout Paris.

This advance selection from the viewpoint of resistance undoubtedly explains the rather high percentage obtained (12.1%). However, this figure, as Chabbert notes, "prevents us from being absolutely optimistic on the future of the resistance of staphylococci to methycillin and to methyl-phenyl-isoxazolyl-penicillin."

(B) Semi-Synthetic Penicillins with Spectrum Identical to That of Penicillin G, But Stable in Acid Medium

These products have an advantage over penicillin G in that they can be used orally.

Their superiority is not as great as that of natural penicillin V ( $\alpha$ -phenoxy-methyl-penicillin) which likewise resists stomach acidity and which has for a long time been used buccally in therapy. Although the blood rates [counts] obtained with the semi-synthetic derivatives, are higher, their antibacterial activity for a given concentration is generally weaker than that of penicillin V.

The advantage of high seric concentrations however often compensates for this inconvenience.

Phenothicillin (  $\alpha$ -phenoxy-ethyl-penicillin) was the first semi-synthetic penicillin in this category to be used in France.

Jeune and Humbert indicated that they were satisfied with its use in the treatment and prevention of scarlet fever.

The usual posology is 1.50-2 g in adults, in 3 or 4 daily administrations. It is cut in half for children.

Propicillin (  $\alpha$ -phenoxy-propyl-penicillin) has been very well absorbed and likewise gives us high blood rates but it is likewise rapidly eliminated.

The usual posology here is 2 g in 24 hours for adults and in 3 or 4 daily administrations.

Clometocillin (  $\alpha$ -methoxy-dichlorobenzyl-penicillin).

The minimum inhibiting concentrations obtained for various strains are rather weak and rather close to those of penicillin G and V.

Furthermore, the serum titers obtained by the usual posology are high (particularly in children) and also relatively prolonged since they are still effective 8 hours after ingestion.

Its use therefore looks particularly promising.

The usual posology is 1 g in 24 hrs for adults, taken 2 or 3 times, and 50 cg to 1 g in 2 or 3 administrations in children.

Generally speaking, the semi-synthetic penicillins in this category should be used only for benign infections or infections of medium seriousness. One of their best indications seems to be rhino-pharyngeal infections. They can also render excellent service in prevention of streptococcus in acute rheumatism of the joints as well as in cases of glomerulo-nephritis.

Digestive tolerance appears to be good. However, it is advisable to be rather cautious if the patient reveals irregular features in his elimination (such as diarrhea).

(C) Semi-Synthetic Penicillin with Spectrum Enlarged to Include the Gram-Negative Bacilli

Right now, there is only one product (D-alpha-amino-phenylacetamide-penicillin or Ampicillin) revealing this property which is rather new for a penicillin, in other words, the new aspect here is the reaction with gram-negative bacilli.

It furthermore offers the advantage of being stable in an acid environment and of being active when administered buccally.

With respect to gram-positive cocci, ampicillin is only slightly less active than penicillin G.

It is inactive with respect to staphylococci which secrete penicillinase because it is sensitive to that enzyme.

It is very active against *Haemophilus influenzae* (the Pfeiffer bacillus) and against the *Neisseria*.

But the most original activity is the activity which is manifested with respect to the gram-negative bacilli of the family of the *Enterobacteriaceae*.

The results have turned out to be excellent with respect to all of the strains of *Salmonella* that were tested; their multiplication is in effect inhibited by concentrations averaging between 0.25 and 1.25  $\mu\text{g}$  per ml, concentrations which are smaller than those of tetracyclin and chloramphenicol.

The activity is likewise good with respect to the strains of *Shigella* tested.

It is very irregular with respect to the coliforms (*E. Coli*, *Klebsiella*, *Aerobacter*) and *proteus*.

Indeed, although most of the strains of coliforms and *proteus* proved to be sensitive to utilizable concentrations, some of them nevertheless do resist. These undoubtedly are strains which secrete penicillinase.

With respect to sensitive strains, ampicillin offers the advantage over tetracyclin and chloramphenicol in that it is bactericidal at concentrations very close to the bacteriostatic concentrations.

Here, in the area of gram-negative bacilli, we thus find one of the biggest advantages of all of the penicillins.

We must however remember that other factors play a role in antibiotic activity, for example, the in vivo concentrations obtained in one part or the other of the organism. Thus the good activity of chloramphenicol in typhoid fever has been attributed to its strong concentrations in the lymphatic system.

Finally, ampicillin is entirely inactive with respect to the pyocyanic bacillus.

Ampicillin is quickly and completely absorbed by the intestinal mucosa. The blood titers obtained are at a maximum after about 2 hours and they are greatly diminished after about 4 hours.

Elimination is essentially urinary and biliary. Indeed, heavy concentrations are found in the urine and in the bile, which may be useful in therapy. The diffusion into the cerebrospinal liquid is very weak.

# SEMI-SYNTHETIC PENICILLINS CURRENTLY SOLD IN FRANCE

Common name, chemical designa- tion	Commercial brand name in France	Behavior toward penicil- linase	Method of admini- stra- tion	Theoret- ical anti- bacterial spectrum	Therapeutic indica- tions (schematic)
Methycil- lin	Flabel- line Penistaph	Resist- ant	Parenter- al	Identical to penicil. G (cocci gram + & gram - & bacilli gram +)	Staphylococcias due to strain secreting pen- icillinase (re- sisting penicil- lin G)
Oxacillin (5-methyl- 3-phenyl-4 isoxazolyl- penicillin)	Bristo- phen	Resist- ant	Oral Paren- teral	same	same
Phenathi- cillin ( $\alpha$ -phenoxy- ethyl-peni- cillin)	Synthe- cillin Peniplus	Sensi- tive	Oral	same	Infections due to cocci with minor or aver- age seriousness. Prevention of streptococcias.
Propicillin ( $\alpha$ -phenoxy- propyl-peni- cillin)	Domocil- lin	Sensi- tive	Oral	same	same
Clometocil- lin( $\alpha$ - methoxy- dichloro- benzyl- penicillin)	Rixapen	Sensi- tive	Oral	same	same
Ampicillin (D- $\alpha$ -amino- phenyl-acetamido- penicillin)	Penicline	Sensi- tive	Oral	Expanded to cer- tain gram- nega- tive bac- illi	Infections due to cocci; in- fections due to enterobacteria- cae (Salmonella, shigella, coli- bacilli, proteus) Infections due to Pfeiffer.



The first clinical uses have produced satisfactory results, particularly in cases of urinary infections due to colibacilli, proteus, and enterococci; however, this is also true of general infections, such as the Salmonellosis or broncho-pulmonary super-infections due to Pfeiffer bacilli. Success has also been reported in cases of septicemias due to gram-negative bacilli and endocarditis.

The usual posology is 1.50 g - 2 g every 24 hrs, in 4 administrations, for adults, and about 125 mg per kg of weight and every 24 hours for children, likewise 4 times.

Digestive tolerance is good; however, in view of the broad spectrum of ampicillin, it is theoretically advisable to watch out for the appearance of an enterocolitis due to resistant germs (for example, a staphylococcus that secretes penicillinase) as a result of the substitution of flora, as for all of the oral antibiotics with a broad spectrum. We will therefore have to be cautious in our posology and in the duration of treatment and we will have to watch intestinal passage.

The table above summarizes the characteristics of these semi-synthetic penicillins.

Abu-Nassar H; Williams, T.W.; You, E.M: Comparative laboratory and clinical observations on two new oral penicillins, oxacillin and ancillin, Amer J Med Sci, 1963, 245, 459-466.

Bunn, A; Amberg, J; Initial Clinical and Laboratory Experiences with Methyl-phenyl-isoxazyl-penicillin, New York State J Med, 1961, 61, No 24, 4158-4162.

Chabbert, Y.A; Baudens, J.G; "Strains of Staphylococci Which Naturally Resist Methicillin and 5-methyl-3 phenyl-4 isoxazolyl penicillin (P.12)," Ann Inst Pasteur, [Yearbook of the Pasteur Institute], Vol 103, No 2, 1962, pp 222-230.

Jeune, M; Humbert, G; "Phenethicillin in the Treatment and Prevention of Scarlet Fever," Pediatric (Lyon) [Pediatrics], 1962, No 8, 915-919.

Leduc, A; Fontaine, J; "In vitro and in vivo Studies of a New Synthetic Penicillin, 5 methyl-3 phenyl-4 isoxazolyl-penicillin, The Canadian Medical Association J, 1962, 86, 101-105.

Martin, M, et al; "Septicemia Due to Staphylococcus Which Resists Current Antibiotics -- Purulent Fusion of the Spleen -- Recovery After Splenectomy and Massive Therapy Using Dimethoxy-benzamide-penicillinate of Sodium," In Presse medicale [The Medical Press], Vol 70, No 16, 1962, pp 771-772.

Massias, P; "Use of a New Synthetic Penicillin in Surgical Staphylococci -- Oxacillin," La Gaz Med de France [The Medical Gazette of France] Vol 70, No 13, 1963, pp 2357-2361.

Rolinson, G.B.; Stevens, S; Microbiological Studies on a New Broad-Spectrum Penicillin, "Penbirin," British Med J, 1961, 2, 5246, 191-196.

Rutenburg, A.M.; Greenberg, H.L; Levenson, S.S; Schweinburg, F.B; Clinical Evaluation of 5-methyl-phenyl-4 isoxazolyl penicillin in Staphylococcal Infections, New England J of Med, 1962, 266, No 15, pp 755-758.

## 2. Antibiotics of the Streptogramin Group (Compound Antibiotics)

In 1961 we reported the first results published on biological and clinical experimentation with staphylomycin.

The ease with which we can use this antibiotic, the absence of toxicity, and the fact that a very small number of staphylococci resist -- these have never been denied.

Another substance of the same group was sold in 1962. This is Pristinamycin (Pyostacin) which was isolated in France from one of the streptomycetes (*S. pristinae spiralis*).

This again is a mixture of several substances (at least two) whose chemical nature is not entirely known.

Pristinamycin exerts its activity with respect to gram-positive and gram-negative cocci as well as gram-positive bacilli. On the other hand, it is inactive against gram-negative bacilli.

As in the case of staphylomycin, most of the staphylococci strains are sensitive here.

The bactericidal concentrations seems to be rather close to the bacteriostatic concentrations. There is a cross-bred reciprocal resistance between staphylomycin and pristinamycin.

The toxicity is practically zero. The blood concentrations obtained experimentally in the serum after administration of the usual dose are at a maximum after about 2 hours and they are greater than the inhibiting concentrations observed in vitro.

The concentration in the organs is the same as that in the blood.

Many therapeutic successes have been reported, particularly in staphylococcosis. For instance, Darbon had 5 definite successes out of 7 cases of septicemia due to staphylococci. In the surgical staphylococcosis and particularly in the osteo-articular ones, favorable results were likewise obtained (Darbon, et al).

Because of its good activity with respect to gonococcus, Siboulet proposed a "minute" treatment of acute bleenorragia by a single dose of 2.5 g (pristinamycin is not active against treponema).

In infections of medium seriousness, the posologies are between 2-3 g every 24 hrs, distributed over 3 or 4 doses in the adult; and 50-100 mg per kg, every 24 hrs for children.

A series of studies concerning this antibiotic was published in La Semaine des Hopitaux [Hospital Week] (Therapy Week), No 38, 1962.

### 3. The Rifamycins

The rifamycins are antibiotics that were isolated in Italy from a strain of streptomyces (*streptomyces mediterranei*).

Rifamycin SV (Rifocine), which seems to be the most active here, is a chemical derivative of natural products.

A recent symposium (Milan, June 1963) brought out the fact that pharmacological, bacteriological, and clinical tests have been made with this antibiotic.

It is used in therapy using the parenteral method.

The minimum inhibiting concentrations -- with respect to staphylococci, streptococci, and pneumococci -- are among the lowest ever observed.

Staphylococci strains which resist spontaneously are very rare today (Hauduroy); there is reason to fear, however, that they will become increasingly frequent as we go along and as the employment of antibiotics becomes increasingly widespread. There is no cross-bred resistance with the other antibiotics.

The enterococci, the gram-negative diplococci, and the gram-positive bacilli have their multiplication inhibited by rather low concentrations.

The bactericidal concentrations are likewise rather weak.

The bacteriostatic activity upon the gram-negative bacilli on the other hand is much less marked.

One particular feature which we might point out here and which might be interesting is the good bacteriostatic activity of rifamycin SV with respect to mycobacteria and particularly the Koch bacillus.

A pharmacodynamic study has revealed that -- after the intramuscular injection of the doses used in therapy (250-500 mg) -- the blood titers went up to as much as 2-6 mg per ml, about 1 hour after injection. Therapeutic concentrations can still be observed during the 8th hour.

The mode of excretion of rifamycin SV is very special. This antibiotic is in effect eliminated essentially by the liver and the bile (60-80%) where very heavy concentrations can be observed (more than 1 mg/ml).

Renal elimination is much weaker. The product is not diffused into the LCR. The therapeutic indications thus are essentially those for cocci infections, particularly infections due to staphylococci that resist the other antibiotics, and the hepato-biliary infections, likewise due to gram-negative bacilli, because the biliary concentrations generally are greater than the minimum inhibiting concentrations observed with respect to these bacteria in vitro.

The clinical experimentation here was accomplished especially in Italy. Many therapeutic successes have been reported particularly in medical or surgical staphylococci and in cases of cholecystitis and angio-cholitis.

The use in tuberculosis is now being studied.

The tolerance of the medication seems to be excellent; in particular, we do not observe any toxicity with respect to renal, hepatic, and hematopoietic functions. The absence of toxicity for the 8th pair [couple] has been emphasized. A few cases of skin intolerance have been reported.

The intramuscular injections are well supported locally although they are sometimes painful.

The usual posology is 0.50 g-1 g distributed in 2 or 3 intramuscular injections every 24 hours in adults. In case of children, we use doses between 0.125 g and 0.75 g.

Tapie, P; La rifamycine. Contribution a son etude experimentale /Rifamycin -- Contribution to Its Experimental Study/, Thesis, Paris, 1962.

Hauduroy, P, Rosset, W; "Sensitivity of Staphylococci to Rifamycin. SV," Chemotherapia [Chemical Therapy], 1963, 6, pp 315-318.

#### 4. Fusidic Acid (Fucidin)

This was isolated in Denmark from a "Fusidium coccineum" mushroom.

Its chemical structure is steroidal. In therapy, we use the sodium salt of this acid. Much experimentation was done on this product in the Scandinavian countries and Great Britain. In France, experiments were conducted by Martin, Chabbert, and their associates.

The spectrum of antibacterial activity, as in the case of penicillin, extends to the gram-positive and gram-negative cocci and to the gram-positive bacilli.

The activity is particularly interesting with respect to staphylococci. The minimum inhibiting concentrations, capable of causing bacteriostasis, are low and somewhat close to those for penicillin G (for sensitive strains). Bacteriostasis is inhibited in the presence of serum (25%)

because of a connection [bond] with the proteins which, furthermore, is reversible.

The bactericidal concentrations are definitely higher than those of penicillin. There is no cross-bred resistance with the other antibiotics, except with cephalosporin P which is likewise an antibiotic with a steroid structure.

RIGHT Now, the number of staphylococcus strains which resist fucidin is very small but the generalized use of this antibiotic involves the risk of causing a selective development of these strains.

To avoid the selection of resistant mutants, it seems a good idea to use fucidin in association particularly with the penicillins, especially since this association is considered synergic by most of the authors from the viewpoint of bacteria-killing.

The blood concentrations obtained with therapeutic doses of fucidin are high, something like 15-20 mug per ml.

They can even attain definitely higher rates (more than 100 mug/ml) after continuous treatment because of an accumulation in the blood.

The excretion is rather slow and takes place through the bile and the urine. Urinary elimination is weak.

The concentration in tissues is weaker than in the blood.

The diffusion into the cephalorachidian liquid is rather high (1/4 of the blood titer, approximately); this might perhaps make it possible to use fucidin in certain cases of meningitis.

Many clinical experiments have been published. In France, Martin obtained 21 favorable results in 28 cases of staphylococci, particularly in two cases involving osteomyelitis.

Other Scandanavian or British authors obtained good results in septicemias by combining penicillin with fucidin or methycillin-fucidin. The best indications seem to be the chronic staphylococci and those staphylococci where the lesions are well vascularized.

The medication is administered per os, in coated pills, and it is tolerated rather well, apart from some digestive manifestations (gastric burns, intestinal passage disorders). But no toxic accidents involving the liver, the kidneys, the hematopoietic or sensory organs have been reported. No action of the hormone type has been reported for this medication.

The usual posology is 1.50 g-2 g per day, three times.

Martin R; Chabbert, Y.A; Martin, E; Barrod, J; "Fucidin, a New Oral Antibiotic for Action on Staphylococci," La Presse Medicale, 1963, 71, No 2, pp 51-53.

Barber, M.D; Waterworth, P; Antibacterial Activity in vitro of Fucidin, Lancet, 1962, 1, pp 928-933.

Seavon, E.F., Garrod, L.P; A Case of Staphylococcal Septicemia Treated with Penicillin and Fucidin, Lancet, 1962, 1, pp 933-935.

Taylor, G., Bloor, K; Antistaphylococcal Activity of Fucidin, Lancet, 1962, 1, pp 935-937.

##### 5. Kitasamycin or Leucomycin (Synaptin)

This is an antibiotic extracted from cultures of streptomyces (*streptomyces kitasatoensis* hata) in Japan in 1953. It has been used for a rather short time in France.

This is actually a mixture of various antibiotic substances (6) whose chemical nature is not entirely clear; it is part of the group of macrolids (erythromycin, carbomycin, oleandomycin, spiramycin); but, because of its compound nature, it is closer to the antibiotics of the group of streptogramin (staphyloxylin, pristinaamycin).

The kitasamycin base (a white powder with a bitter taste) and acetyl-kitasamycin (insipid) are used via the buccal route.

Tartrate of kitasamycin, which is soluble in water, can be used via the parenteral route, although only i.v., because of its acid pH.

Like that of the macrolids or gram-positive cocci, the antibacterial spectrum extends to the gram-negative cocci (*Neisseria*), to the gram-positive bacilli, and to some of the gram-negative bacilli (*Haemophilus*, *Bordetella*, *Brucella*).

Most of the gram-negative bacilli, on the other hand (*Enterobacteriaceae*, *pyocyanic*) are not sensitive to the utilizable concentrations.

Janbon, et al, tested this antibiotic against 118 strains of staphylococci isolated in their hospital ward.

Of these, 52 strains (44.1%) were highly sensitive but 43 strains were highly resistant (C M I higher than 25 µg/ml).

Other strains were sensitive to intermediate concentrations.

Most of the resistant strains came from subjects who had already been treated with other antibiotics; this suggests a phenomenon of cross-bred resistance.

In vitro, the authors were able to render an initially sensitive staphylococcus strain resistant. This resistance was cross-bred with erythromycin and pristinamycin.

The authors also tested kitasamycin against 20 strains of enterococci. It proved to be more active at the usual concentrations than most of the other antibiotics. The blood concentrations obtained after the usual dose of 400,000 units (400 mg) are at a maximum during the first 2 hours. They reach rates between 0.50 and 1 mg/ml (Ravina).

Clinical studies gave the French researchers a good impression in infections of minor or average seriousness due to pyogens; about 80% success was registered, particularly in skin and lung infections.

This antibiotic is of interest primarily because of its perfect tolerance which makes it extremely easy to handle. This confirms the almost zero toxicity found in animals.

The possibility of using heavy concentrations i.v. will make it perhaps useful in certain serious staphylococci.

The usual posology in the adult is 800,000-1,600,000 units (or 800-1,600 mg) by the buccal route, administered 4 times; 600,000 units or more in i.v. perfusion, for 24 hours. For children, there is one form that comes in a syrup.

"A New Antibiotic, Syneptin (Kitasamycin)," Gazette Med France, Vol 70, No out of series, 1963.

Ravina, A, "Kitasamycin," Presse Med, Vol 71, No 46, 1963, pp 2198-2199.

#### 6. Paromomycin (Humatin)

This was extracted in the United States from cultures of "streptomyces rimosus paromomycinus." Its chemical formula is known (D-glucosamine-desoxystreptamine-D-ribose Diaminohexose). It belongs to the family of oligosaccharides (streptomycin, kanamycin, neomycin, soframycin).

This antibiotic cannot be used parenterally because it is highly toxic for the 8th cranial pair and for the kidney. When absorbed per os, it is practically not absorbed at all and it is used in infections and in intestinal parasitoses.

Its activity spectrum is quite widespread.

In vitro, it inhibits the multiplication of most of the gram-positive or gram-negative bacteria at concentrations which can be obtained in the intestinal lumen.

But -- and this is its original feature here -- it is also very active with respect to the dysenteric ameba because of the direct action which was demonstrated in vitro and which comes in addition to the action upon the associated flora.

An activity with respect to other protozoa (Lamblias, trichomonas) was also discovered.

The clinical tests revealed excellent results in the field of intestinal bacterial infections, particularly the salmonelloses and the shigelloses, intestinal infections due to pathogenic or pyocyanic staphylococci, and in children in the case of gastroenteritis due to enteropathogenic colibacilli.

Much experimentation has been carried on in several countries with respect to its use in amebiasis. In Morocco it gave highly satisfactory results in the acute or subacute forms in the research done by Hugonot and associates. It also produced good results in other intestinal protozooses.

The product is well tolerated. Diarrhea cases attributed to microbial lysis can be observed some times. There is also reason to watch out for diarrhea due to yeast (candida), as a result of flora substitution.

The daily dose is 1-2 g per day for the adults and 30-50 mg/kg for children.

The average duration of treatment is 4-7 days. A single dose of 4 g has been proposed in the case of amebiasis.

Hugonot, R; Jego, J; Latapie, J.L; Foissac-Gegoux, Ph; "Treatment of Intestinal Amebiasis by a Single Administration of Paromycin Sulfate," Bull et Mem Soc Med Hop, Paris / Bulletin and Reports of the Paris Medical Hospital Society, Vol 77, No 7 and 8, 1961, pp 211-215.

## 7. Modified Antibiotics

Other antibiotics recently sold in France are substances that are either related to or that are modifications of existing antibiotics:

Thiamphenicol (Thiophenicol), which we announced in 1961, is now in use in France.

We might remember that this is a derivative of chloramphenicol where the  $\text{NO}_2$  radical situated in a para position on the benzenic nucleus is replaced by a methylsulfone radical ( $\text{CH}_3\text{-SO}_2$ ).

On an experimental basis, in animals, thiophenicol, when administered in equal doses, produced less high blood concentrations although they were more prolonged than in the case of chloramphenicol and urinary



and biliary concentrations which were definitely higher. On the other hand it was slightly less toxic for laboratory animals. From the bacteriological viewpoint, its activity is exerted on the same germs as that of chloramphenicol but it is much weaker. The minimum inhibiting concentrations in effect are generally higher, particularly for the salmonella.

Thiophenicol is used above all in urinary infections and typhoid fever.

It was studied recently by Janbon and associates and by Keddari in connection with typhus. These authors found the following: good activity on the part of the product, since all cases recovered with posologies varying between 5 and 10 cg/kg, on the average; the possibility of relapses (28% of the cases), but the absence of complications connected with bacterial lysis and the liberation of endotoxin -- this, perhaps because of the "gentler" action of thiophenicol which is less bacteriostatic than chloramphenicol; the rapid sterilization of the stools in the cases verified, perhaps due to the better bile concentration; it is difficult to estimate the medullo-sanguinary toxicity. In some cases, a decrease in the granulocyte count was found after treatment, but there was no aplasia.

As far as we know, the literature on this subject does not contain any cases of aplasia connected with thiophenicol. However, in view of the rarity of these accidents, we do not have enough information to come up with a final opinion.

Janbon, M; Brunel, D; Bertrand, A; Alleu, Y; "First Experience in the Treatment of Typhoid Fever with Thiophenicol," Montpellier Med, 40, 1961, 26.

Keddari, M; Le traitement de la fièvre typhoïde par le thiophenicol /Treatment of Typhoid Fever with Thiophenicol/, based on 33 observations collected at the infectious disease clinics, Pr Janbon, thesis, Montpellier, 1963.

Penimepicyclin (penetracycl) is an original antibiotic resulting from the synthesis of two conventional antibiotics -- penicillin V and tetracyclin -- into a single molecule. More specifically, we are dealing here with phenoxy-methyl-penicillinate of a tetracyclin which has been made highly soluble, in other words, mepicyclin (4-hydroxyethyl-diethylenediamino-methyl-tetracyclin).

Penimepicyclin is highly soluble. It can thus be used both parenterally and buccally and it diffuses better into the organism than penicillin V and tetracyclin, by themselves.

The blood titer (expressed in basic tetracyclin) obtained with the usual dose is between 3 and 4 mug/ml. They are at a maximum about 30-60 minutes after the administration (per os, IM) and they persist (12 hours) because of the rather slow renal elimination.

Passage into the cophalorachidian liquid is rather good (1/8 of the blood titers) and permits utilization in certain cases of meningitis.

The antibacterial spectrum is very broad and extends to the cocci and the gram-positive and gram-negative bacilli.

The mepicyclin fraction seems to protect the penicillin V fraction against the action of penicillinase and this explains the action on strains that resist penicillins G and V.

The therapeutic results published in general medicine and in pediatrics are satisfactory.

This antibiotic is extremely easy to handle due to the absence of toxicity and its good tolerance when administered by all routes.

The usual posologies are 1 g in 24 hours, administered twice (this corresponds to 50 cg of tetracyclin and 600,000 units of penicillin V), either orally or IM. For children, we prescribe 15-30 mg/kg and per 24 hours.

These doses can be increased without inconvenience in severe infections.

Intravenous perfusion can also be used then. Local utilization is likewise possible.

Demethylchlortetracyclin (mexocin). This substance, produced by a mutant of "streptomyces aureofaciens" is a part of the tetracyclin group.

Better intestinal absorption and slower renal elimination give it higher and more prolonged blood titers than the other cyclins. A more reduced posology is thus possible (60 cg in 24 hours, administered twice, in the case of adults, 10-15 mg/kg and 24 hours for children). Numerous clinicians have experimented with this product in France. They reported excellent results particularly in oto-rhino-laryngological and respiratory infections in adults and children. Tolerance is very good. We must however watch out for some very rare incidents of acute diarrhea due to flora substitution and photosensitization, as in the case of the other tetracyclins.

Pyrrolidino-methyl-tetracyclin (transcyclin). This is a soluble and stable tetracyclin which can be used intravenously. The blood titers obtained are high and persist after an i.v. injection of 275 mg at therapeutic doses [rates] for 24 hours. It can be used with greater posologies in adults, in serious infections (2 or 3 injections). Clinical experimentation has been reported with good results. For example, Grislain obtained good results in pediatrics (in continuous perfusions or in the form of injections into the perfusion tubing).

The addition of certain carrier substances to tetracyclin, hexameta-phosphate of sodium (\*hexacyclin) or citric ion (\*pancyclin) or phytic

acid (\*ambramycin) enable us to get blood titers greater than those obtained from the basic tetracyclin used by itself.

Hemisuccinate of Chloramphenicol (\*solnicol). This is a soluble derivative of chloramphenicol which can be used parenterally, IM and IV. It can be extremely helpful in serious infections, in special cases of meningitis, when the buccal route cannot be used (coma, digestive intolerance).

New forms of novobiocin are also being sold now: here we have an injectable form (\*injectable cathomycin), a hydrogenated derivative, dihydronovobiocin (\*vulcamycin), which gives us high blood titers after ingestion.

8. Numerous antibiotic associations have also recently been introduced into therapy. We are, personally, not at all in favor of the multiplication of these products because the posology for each one of these antibiotics, whenever two of them are combined, depends on the other associated antibiotic; besides, it is not easy to study microbial sensitivity by the plate method; the association of two antibiotics is not always desirable and, if it is useful, it had better be left to the free choice of the practicing physician; finally there are many more dangers of intolerance.

9. The incidents and accidents in antibiotherapy continue to preoccupy clinicians.

At the last congress of the Association of French-speaking pediatricians, Laplane and associates discussed this question and placed it in the context of the "medication, the terrain, and the person prescribing the medication"; they also described the mechanisms involved in great detail.

There are two types of unpublished incidents which were reported recently:

F. Richet and associates observed neuro-psychic accidents in 3 chronic uremia patients who had been treated with methane-sulfonate of colistin (parenterally, at doses of 3-6 million units in 24 hours). The disorders were of the obnubilation and dysesthesia types, particularly peribuccal and they were also of the agitation, anxiety, hallucination, hypotonia, abnormal movement types; in two cases, this ended in terminal coma.

These are not accidents due to over-dosage as a result of accumulation, nor are these accidents due to endotoxinic shock; according to the authors, this involves a particular susceptibility of patients with chronic uremia to that particular product. We must note that no increase in renal insufficiency was observed.

This latter fact confirms the absence or the very low renal toxicity of methane-sulfonate of colistin which was noted by many authors.

For our part, we always used this antibiotic without any major incidents and with beneficial results.

Vic-Dupont and associates described an accident involving a very peculiar and very deceptive intolerance of methycillin, an intolerance of the pseudo-infectious syndrome type. In three serious cases of infection treated with methacillin I.V., these authors, after several days or weeks of treatment, while the evolution was favorable, observed a rather abrupt return of fever, accompanied by chills, vomiting, headaches, impression of epigastric bar, and resumption of hyperleucocytosis. However, these alarming symptoms, which simulated a return of infectious processes, disappeared rapidly after the methycillin was stopped.

Such accidents, whose mechanism remains unknown, but which is probably of an allergic nature, are particularly worth-while studying because lack of familiarity with them might mistakenly orient the physician toward an infectious etiology and cause him to use methycillin in the treatment whereas such treatment with methycillin should immediately be interrupted.

Laplane, R; Debray, P; Etienne, M; Graveleau, D; "Accidents in Antibiotherapy," XIX Congres de l'association des pediatres de langue francaise III: Les accidents en therapeutique pediatrique /19th Congress of the Association of French-Speaking Pediatricians. III: Accidents in Pediatric Therapy, 16-126; Expansion scientifique Publishers, Paris, 1963.

Richet, G; Ardaillou, R; Sultan, Y; "Neuro-Psychic Accidents in Chronic Urem Cases Treated with Methane-Sulfonate of Colistin," Bull et Mem Soc Med Hop Paris, 1962, No 14, 1199-1205.

Vic-Dupont; Rapin, M; Huault, G; "Accidents Due to Intolerance of Methycillina of the Pseudo-Infectious Syndrome Type," Presse med, 1963, 71, No 6, 271-273.

10. We will not go into the problem of the new sulfamides here; this problem was covered recently in our magazine.

We would like to ask our readers to refer to that particular article.

From the sulfamides we can move on to a new compound in the group of nitrofurans; this is a compound which was recently introduced in therapy and which is called nitrofurazolidine (furoxane); it can be very advantageously used in intestinal infections, particularly infantile gastroenteritis cases.

Bastin, R; Vilde, J.L; "Current State of Sulfamidotherapy in Infectious Pathology," Rev du Praticien (the Year of the Practitioner, 1963), Vol XIII, 31 bis, 128-132.

### "Aerobacter Cloacae" Septicemias

In our general review of 1961, we reported the increasingly widespread occurrence of serious infections due to germs which in the past were identified as saprophytes or slightly pathogenic, such as the proteus, serratia, and especially the pyocyanic bacilli; an entire issue of La Revue du Praticien was devoted to that subject; the report was entitled "Present-Day Bacterial Infections." We might briefly recall the responsible factors here: selection of these resistant strains as a result of the increasingly widespread and sometimes excessive use of the usual antibiotics; circulation of these strains in a hospital environment; increasingly frequent practice of diverse instrumental explorations or revival maneuvers performed on seriously ill patients or on weakly-resistant operation cases -- although rigorous asepsia is not always easy to carry out.

Recently, four cases of "Aerobacter cloacae" septicemias were reported to the Medical Society of the Paris Hospitals.

The germ involved, a gram-negative bacillus of the family of Enterobacteriaceae, until then had only been considered as a rather minor and unimportant saprophyte, particularly in the intestinal mucosa.

The three cases reported by Vic-Dupont, Lissac, and Amstutz occurred almost simultaneously, causing a veritable minor hospital epidemic in three patients who were stricken with severe tetanus, who had been tracheotomized, curarized, and who were on a "blanket" antibiotherapy regime (penicillin-streptomycin).

The symptomatology revealed a combination of a septicemic syndrome, a broncho-pulmonary attack creating suspicion of an acrial entry way (tracheotomy-aspiration) and, in 2 cases, a coma and irreversible collapse, perhaps pointing to a release of endotoxin.

Along with Verliac, David and Hazan we also reported a case involving an 8-year old child, after heart surgery, with placement of an ivalon prosthesis (CIA with abnormal venous return). As in the favorably developing cases of Vic-Dupont, recovery was obtained only after long antibiotic treatment, which was adapted to the particular bactericidal capacity, combined with corticotherapy whose effect was highly beneficial.

Vic-Dupont, J; Lissac, J; Amstutz, Ph; "Three Cases of Aerobacter cloacae septicemia -- the Result of Hospitalism," Soc Med Hopit Paris, 114, No 3, 1963, 241-247.

Bastin, R; Verliac, F; Lapresle, Cl; David, V; Hazan, J; "Aerobacter cloacae Septicemia -- Recovery Due to Antibiotherapy and Corticotherapy," Soc Med Hopit Paris, 1963, 114, No 3, 249-254.

## Immunofluorescence in Infectious Pathology

The technique of immunofluorescence (IF) is based on the possibility of combining the globulin-antibodies with a fluorochrome, without altering their immunological reactivity; this possibility was discovered by Coons.

Long used almost exclusively in basic research, it was applied more recently in medical diagnosis, particularly in infectious pathology.

A number of variations of this technique have been used:

The direct method consists in establishing contact with a preparation fixed on a slide and presumed to contain the antigen to be identified, on the one hand, and a solution of corresponding antibodies which had earlier been combined with a fluorochrome (generally, isothiocyanate of fluorescein). The antibodies are fixed on the antigens; the non-fixed surplus is eliminated by washing. The preparation is then examined under the microscope, with the black background illuminated by radiation capable of exciting the fluorescence of the fluorochrome (violet, ultraviolet). The antigens are thus made visible by the specific fluorescent antibodies which are fixed on them and they can thus be identified right there. Bacilli will appear as greenish rings; viral antigens will be located inside the infected cells, for example. The preparations are made with the help of various specimens (throat, stool, LCR, histological sections).

The indirect method is generally used to establish the presence of antibodies in the serum of the patient, something like a serum diagnosis or a complement deviation reaction, for example. It is based on the principle of Coombs [sic].

We put the serum from the patient (who was presumed to have syphilis in the example selected here), in a suitable dilution, on a preparation that is fixed on a slide containing known antigens (for example, syphilis treponemas). After washing, which eliminates the surplus of antibodies that are not fixed on the antigens, we, for the second time, place an antibody solution on this and this time the antibody solution is combined with human antigamma globulins which are fixed in turn on the patient's antibodies ("double layer" method). Once again the surplus is eliminated by washing. The antigens appear fluorescent under the UV microscope when the patient's serum contains antibodies; in the opposite case, they remain invisible.

Other methods are less utilized, such as the anticomplementary method or the fluorescence inhibition method.

The solutions of antibodies used are the globulinic fractions of the serums from hyperimmunized animals, combined with fluorochrome under well-determined physicochemical conditions. They can be stored in a refrigerator.

Thus the IF technique features the benefits deriving not only from the specificity of a serological method but also the benefits deriving from the rapidity of microscopic examination.

Among the applications of the IF method in infectious pathology, we must distinguish applications in the field of research, for example, the study of the localization of antigens in the infected cells, the study of the formation of antibodies, the study of the development of antigens in vivo, etc; these are tremendously important from the theoretical viewpoint of course; and we also have applications in the practical field of medical diagnosis which we can quickly review.

Among the enteral infections, only the infantile gastroenteritis cases (GEI) due to pathogenic colibacilli have benefited from this technique. This is a direct method employing combined serums of rabbits, corresponding to the 10 responsible serotypes. The results published by Whitaker (1958), and particularly those published by Le Minor, Fournier, and Eliachar in France (1962) were better than those obtained with the usual technique (culture-agglutination), in terms of sensitivity; as far as the specificity is concerned, it is satisfactory, undoubtedly because of the B antigens which envelop are found in the envelop of these colibacilli.

The rapidity of the method makes it possible to discover infected infants immediately.

Attempts to use them in connection with salmonella, shigella, and cholera vibrio, on the other hand, failed because of the antigenic O communities of these germs with numerous saprophyte bacteria in the digestive tract.

The application of the direct method to the diagnosis of whooping cough was proposed by Donaldson (1960) and Kendrick (1961). In France, J. Marie, Herzog and Gaiffe (1963) were satisfied with a method which enabled them to detect the disease early in a high percentage of cases.

However, certain difficulties, which we likewise encountered, such as the cross-bred reactions with neighboring germs, as well as difficulties of interpretation of certain slides (specimens taken from the throat), have been reported, by the abovementioned authors and undoubtedly explain the possibility of some errors.

The application in the diagnosis of diphtheria has been tackled in two different ways although, in both cases, this involved a direct method applied on scrapings taken from the throat.

Whitaker (1961) combined a diphtheria antitoxin, whereas Moody (1963) used a combined antibacterial serum (prepared through hyper-immunization of a rabbit with the help of antigenically complete live diphtheria bacilli).

These two types of combined substances enabled their users to establish the presence of Klebs-Loeffler bacilli in the specimens taken from the diphtheric throat (allowing the swab to incubate in a liquid medium for several hours prior to the preparation of the smears).

However, neither of these two combined substances produced perfect specificity, it seems, because false positive fluorescences are possible on the part of the saprophyte *Corynebacteriaceae* or other bacteria present in the specimens taken from the throat.

The IF method thus does not appear to offer sufficient reliability in this case, at least it is not far enough along to be used at this time.

Next, it would be logical to take up the topic of direct immunofluorescence for the isolation -- from the throat -- of streptococci of Group A, the only ones that are liable to be responsible for nephritogenic anginas, scarlet fever, R.A.A. [sic], since the serum precipitation method of Lancefield is rather long and delicate.

However, Moody and associates first of all combined an antiserum of Group A and then observed a strong cross-breeding reaction which was quite unexpected and which involved the C and G streptococci here. The extraction of the antiserum and the use of the fluorescence inhibition method reduce this inconvenience.

The results on specimens taken from the throat were reported by several authors (Redys, Peeples, Cherry, Wolfe, Halperen, Warfield) and in 75% to 95% of the cases they agree with the usual method (culture-serum precipitation). The disagreements seem to be due to false positive fluorescences.

Although this application is quite worth-while, it has not yet been sufficiently utilized to enable us to come up with a conclusive judgement as to its value.

Still talking in terms of bacterial infections, a number of other applications have been proposed but the advantage of the IF method over the usual methods is not evident in these cases:

The pathogenic staphylococci which secrete coagulase could be distinguished by a specific conjugate [combined substance] (Carter, Blobel).

Using the direct IF method, Page was able to establish Pfeiffer bacilli in the LCR of meningitis cases due to that virus, even when the culture remained negative after antibiotic treatment.

The possibility of using immunofluorescence in order to identify meningococci and *Listeria monocytogenes* has also been demonstrated.

We might also mention the applications proposed for infections involving brucella; pasteurella; anthrax; swine rotlauf bacilli; melioidosis;



the leptospira; and the Rickettsia.

We might briefly recall here the most worth-while applications of the IF method in the diagnosis of venereal diseases proposed by Deacon; we will not go into detail because this is not exactly our subject here today.

The direct method used in blennorrhagia does not offer any advantages over the simple gram-staining method in acute cases and it gives the same results as the culture in chronic cases, with a certain advantage in terms of simplicity and rapidity (although the swabs must be incubated 12 hours).

In the field of syphilis, the IF method has been used more widely. It was introduced into France by Borel and Pirel (1959). This is an indirect serological method using treponemic antigens.

The antibodies which were established in the serum of patients are not the reagins but rather specific antibodies of TP and the antiprotein of the group (Pillot and Borel, 1961).

Its specificity and importance have been widely debated.

The diagnosis of atypical primitive lung diseases due to the Eaton agent (PP10) seems to benefit considerably from immunofluorescence which, in effect, is today the only practical diagnosis method.

Here again we use the indirect method since the antigen consists of histological sections from chicken embryo lungs experimentally infected with the mycoplasma. The specific antibodies evidenced here in the serum of patients are different from the cold agglutinins. In France, Thivolet, Schier and their associates, working with a series of 102 atypical pneumonia syndromes, were thus able to evidence this etiology 30 times.

In the area of virus infections, diagnostic applications are much more limited.

The diagnosis of grippe by the direct method involving throat washing liquid was proposed by Liu but has produced some highly inconstant results.

It is possible to identify the herpetic virus in skin lesions (Biegelsein).

The rapid identification of poliomyelitis viruses on cell cultures was proposed by Hatch.

The application in the diagnosis of rabies seems to be most worth-while here. The virus antigens can also be established in the nerve cells of infected animals with greater sensitivity than the conventional technique of Negri body staining (Anastasiu; Goldwasser; Etchebarne).

In parasitology, applications in connection with candidosis, smiliasis, trypanosomiasis, helminthiasis, and trichomonas have barely been started.

On the other hand, the indirect method enabled Goldman and, in France, Garin and Ambroise-Thomas, to perform a serum diagnosis of toxoplasmosis as sensitive and reliable as the lysis test.

Overall, the different applications which we have just reviewed are so far only in the exploratory stage. By and large, the speed and sensitivity of immunofluorescence constitute the major advantages here. The reproductibility and specificity of this method on the other hand often are rather approximate.

We must not be too optimistic as to the good that can come of this technique in the field of infectious disease diagnosis.

Le Minor, L; Fournier, P.J; Eliachar, E; "Rapid Detection of E. coli Enteropathogens in Infants by the Immunofluorescence Method," Sem Hopitaux (Annales de pediatrie) / Hospital Week (Pediatrics Yearbook), 38, 1962, 3073.

Marie, J; Herzog, F; Gaiffe, M; "The Rapid Diagnosis of Whooping Cough by the Fluorescent Antibody Technique (100 Initial Results)," Sem Hopitaux (Annales de Pediatrie), 39, 1963, 269-272.

Moody, M.D; Jones, W.L; Identification of "Corynebacterium diphtheria" with Fluorescent Antibacteria Reagents," J. Bact., 1963, 86, 285-293.

Moody, M.D; Siegel, A.C; Pittman, B; Winter, C.C; Fluorescent Antibody Identification of Group A Streptococci From Throat Swabs, A.J. Public Health, 1963, 53, 1083-1092.

Thivolet, J; Schier, R; Picard, J.P; Blanc, G; "Application of the Immunofluorescence Method in the Immunological Diagnosis of Lung Diseases Due to Mycoplasma of Enston," Ann Inst Pasteur, 1963, 105, 749-756.

Anastasiu, P; Lepine, P; Dragonas, P; "Kinetic Study of Rabies Virus in Tissue Culture with the Help of Fluorescent Antibodies and Ultra-Fine Sections," Ann Inst Pasteur, 1963, 105, 813-824.

Garin, J.P; Ambroise-Thomas, P; "The Serological Diagnosis of Toxoplasmosis by the Fluorescent Antibody Method (Indirect Technique)," Presse med., 1963, 71, 2485-2488.

For the bibliography of earlier publications, see the following:

Nairn, R.C., Fluorescent Protein Tracing, 1962, Livingstone, E.S. Ltd, Edinburgh and London.

Eastin, R., Lafaix, Ch; "Immunofluorescence in Infectious Pathology," Journées médicales de Broussais-La Charité / Medical Conferences in Brussels -- Charity Hospital, May 1964, Expansion scientifique, publishers (in print).

#### Clinical Data on Infectious Mononucleosis

The atypical forms of infectious mononucleosis can be connected with their etiology by means of the serological reaction of Paul Bunnell and Davidsohn. A number of recent publications also reported observations on rare locations of this disease.

Verliac and associates in 1962 observed two severe cases of neurological location in young subjects.

In the first case, encephalitis appeared after a characteristic angina, without any meningeal reaction leading to a coma with convulsive crises and developing toward death in spite of attempts at revival.

The blood formula was not at all characteristic but the Paul Bunnell-Davidsohn reaction was quite positive.

The second observation involves a serious case of meningomyelitis expressed by paraplegia with hypercytosis and rachidian hyperalbuminosis, in the course of a rather minor febrile angina.

The hemogram and the serology were characteristic of infectious mononucleosis. Recovery was achieved although a number of sequelae persisted (genito-urinary disorders, Babinski sign).

At the same session of the Medical Society of the Paris Hospitals, Mahoudeau reported a case of peripheral facial paralysis in the course of infectious mononucleosis.

Neel and associates reported three cases of neurological forms. These involved one case of primitive lymphocytary meningitis, one case of secondary lymphocytary meningitis in the course of development of infectious mononucleosis, and one case of meningo-encephalitis with a disturbed electro-encephalogram.

All of these patients recovered without any sequelae. The etiology was demonstrated by serology.

Mertenova and associates reported one case of infectious mononucleosis beginning with a coma.

The frequency of nervous complications in infectious mononucleosis was about 1%. This essentially involved -- in order of decreasing frequency -- meningeal reactions, polyradiculoneuritis, and encephalitis.

The classically favorable prognosis has not always been found to bear out the observations of Verliac.

Their treatment seems to benefit from corticotherapy associated with antibiotherapy as well as, possibly, techniques of respiratory resuscitation (Grenet).

Albeaux-Fernet and associates reported another rather minor location of the disease since they found only about a score of published observations on this. This involved an acute febrile pericarditis, accompanied secondarily by edema of the face, diffuse adenopathies, and splenomegaly. The hemogram and the Paul Bunnell-Davidsohn reaction provided the etiology of the disease.

Gregoire and associates, finally, mention a genital location of the balano-posthite erosive type, accompanied by fever, asthenias, adenopathies, and erythema. The hemogram was characteristic and the serology was positive.

The case developed toward recovery. The authors only found three comparable observations in literature.

Facts such as these, and the inguinal ganglionic forms described by Chevalier suggest the possibility of a mode of venereal contamination through salivary contact, as emphasized by Lepine.

All of these rare forms of infectious mononucleosis should certainly cause the practitioner to perform a serological Paul Bunnell-Davidsohn reaction, in view of the entire unusual symptomatology associated with blood mononucleosis (with basophile elements); the practitioner must realize that it develops positively between the 8th and 14th days of the disease and that it turns negative around the 3rd month.

The other reactions proposed, which by the way are not currently used, do not seem to be preferable here (Eyquem).

Verliac, F; Lamelin J.P; Magloire, C; "Two Unusual Complications of Infectious Mononucleosis -- Deadly Encephalitis and Meningo-Myelitis," Bull et Mem de la Soc Med Hop Paris, 1963, 114, No 2, 179-184.

Neel, J.L; Groussin, P; "The Nervous Forms of Infectious Mononucleosis (In Connection With Three Cases of Meningial and Meningo-Encephalitic Forms)," Le Concours Medical / The Medical Concourse, 1962, No 51, 7003-7006.

Mortanova, J, et al; "Comatose States in the Course of Infectious Mononucleosis -- Report on a Case History," La Presse med, 1962, 70, No 22, 1081-1082.

Albeaux-Fernet, M; Chabot, J; Moreau, Ph; "Acute Pericarditis -- Initial Manifestation of Infectious Mononucleosis," Bull et Mem de la Soc Med Hopit Paris, 1962, 113, No 13, 105-1059.

Gregoire, J; Labram, C; Dreyfus, B; Domart, A; Hazard, J; "A Case of Infectious Mononucleosis with Genital Location," Bull et Mem de la Soc Med Hopitaux de Paris, 1962, 113, No 4, 283-290.

Lepine, P; "Etiology and Epidemiology of Infectious Mononucleosis," Rev du Praticien, 1962, 12, No 25, 2667-2673.

Grenet, P; Chavelet, F; de Prillerets, F; "Infectious Mononucleosis," ibid, 2675-2685.

Eyquem, A; "The Paul-Bunnell and Davidson Reaction," Ibid, 2703-2711.

What Is the Place of Sudden Exanthema Among the Erythematous Viroses?

Among the erythematous viroses in children, sudden exanthema which...  
[rest of paragraph illegible in photostat].

Recently, Monnet and ...[rest of line illegible in photostat], in their study of 42 cases, and Bernard, discussed this problem.

Sudden exanthema is essentially an infant's disease; it usually attacks infants between the ages of 3 months and 2 years; out of the 42 infants observed by Monnet and Fleurette, none was more than 2 years old.

The frequency of this disease is undoubtedly very great but, most of the time it is not recognized and it may sometimes even be not at all apparent.

The disease is defined by its rather peculiar symptomatology.

It manifests itself first of all by a generally rather steady fever, which most often is quite high (39° C) and which lasts 3 days. During that time the infant is agitated, cranky, and rather sleepless. The physical symptomatology is very poor: the throat and the tympanums are congested; sometimes there are small adenopathies in the neck and their may also be a discreet splenomegaly.

The eruption appears only on the 4th day, after thermal defervescence. It is always quite discreet and rather fleeting, it consists of generally separated and sometimes confluent pale-pink maculo-papules which settle on the neck and the trunk.

The delayed appearance of a rather fleeting eruption explains the fact that the disease is frequently not identified as such.

During that period, the hemogram will constantly reveal leucopenia with granulopenia.

Sometimes, complications can be observed. Joseph and associates several years ago emphasized the nervous complications of the disease.

These were encephalitic manifestations of the convulsion type, with motor deficiencies; a discreet clinical or biological meningeal syndrome can likewise be observed. These alarming signs generally are completely regressive. In some cases, however, one can note accompanying sequelae.

Digestive disorders are also possible.

The work done on the etiology of the 6th disease actually amounts to very little.

Certain authors (Shaw, et al) were able to transmit the disease to monkeys; Fleurette observed a cytopathogenic effect after planting specimens on human kidney cell cultures.

However, the question as to whether sudden exanthema is a disease due to an as yet unknown virus or whether we are dealing here with a syndrome corresponding to several viral etiologies -- that question has not yet been solved.

Future virological studies will perhaps make it possible to answer this question with certainty. Speaking more generally, the chapter of erythematous viroses is full of surprises.

As a matter of fact, only two diseases have been perfectly defined, both from the clinical viewpoint and from the virological viewpoint; they are: measles and German measles, whose viruses were isolated, respectively, in 1954 and 1962.

Among the others we must distinguish the following:

Old clinical framework: the 4th disease (Filatov, 1886, Dukas 1900); the 5th disease or epidemic megalerythema (Sticker 1899); sudden exanthema (Zahorsky, 1910).

But, among these, the first of the three mentioned here does not correspond to anything precise, the 2nd has a rather disputed nature, and only the 3rd, as we have just seen, gives us a clinically well-defined picture although the etiology likewise is not precisely spelled out.

#### Recent virological acquisitions.

In recent years it has been discovered that certain recently isolated viruses were capable of producing erythematous eruptions. For example: Echo viruses, particularly Echo 9 and Echo 16 (Boston exanthema); the coxsackie A or B virus; more rarely, certain types of adenovirus, etc.

Actually, right now it is difficult to assemble these various differing facts in a precise fashion. It might be a good idea to draw up a

new classification in the light of advances in virology which will make precise biological diagnosis possible.

Monnot, P; Fleurette, J; "The 6th Disease or Sudden Exanthema," Sem Hopitaux (Ann Pediat), 1963, 39, No 23, 190-195 (802-807).

Bernard, R; "Infantile Roseola," Marseille Medical, 1963, 100, No 5, 321-326.

Joseph, R; Ribierre, E.M.; Job C; Gabilan, J; "Nervous Complications Due to Sudden Exanthema," Sem Hopit (Ann Pediat), 1958, 34, No 9, 554-559.

#### Recent Discoveries on German Measles

German measles is a disease which is almost always benign in children or adults.

The incidence of encephalitis due to measles is extremely low. However, in connection with the publication of three serious forms which were observed during a British epidemic in 1962, Pampiglione and associates noted that a rather rough attack on the brain is undoubtedly more frequent than one generally assumes. In effect, they also reported seven cases of slight encephalitis and they point out that the EEG's, which they took systematically, were disturbed not only in these cases of encephalitis but in many cases of measles without any other complications.

As for the death rate due to encephalitis, this was evaluated by Miller who reviewed 80 cases in medical literature in 1956 and found 20% deaths.

But above all, as we know, ever since the first observations by Gregg, in Australia, in 1941, it is measles which generates embryopathies during the first weeks of pregnancy, if it strikes receptive pregnant women, that is to say, women who have not had German measles before.

Abortions and congenital malformations are the consequences of this. The latter vary according to the state of development of the embryo at the moment of its transplacental contamination and they may be diversely associated. This essentially involves eye and heart malformations, deafness-dumbness, psycho-motor retardation with or without microcephaly.

The frequency of these embryopathies in pregnant women who contract the disease during the first 3 months of pregnancy appears less high than it seemed at first sight. The 50-80% figures given in the first studies (Parsons, Swann, Ingalls, Ober, and in France, Lamy and Seror) seem to have been out back in the light of more extensive and more recent statistics, down to about 10-35% according to the following authors (in Sweden -- Lundstrom; in the US -- Siegel and Greenberg; in Australia -- Pitt; in Great Britain -- Manson; and in New Zealand -- Liggins). On the other hand,

after a recent survey in Great Britain conducted by the College of General Practitioners on 510 cases of German measles and on contagiousness in the surroundings, only 3.7% of the women of child-bearing age contracted the disease, undoubtedly because of immunity acquired in childhood.

An embryopathy might thus manifest itself in 1%, roughly, of pregnant women exposed to contagion at the beginning of their pregnancy. This figure is still unfortunately very high and an effective means of prevention would certainly be most useful here.

Right now, passive immunization is the only method possible. It employs above all the standard gamma globulins whose effectiveness does not seem to be any less than that of the gamma globulins or whole serum from convalescents (Lundstrom).

This means of protection looked effective to most of the statisticians (Lundstrom, Soulier). Very recently, MacDonald contributed the results of a very broad systematic prevention campaign for pregnant women, involving standard gamma globulins; this campaign was conducted in Great Britain between 1954 and 1961 (16,121 doses were administered); it appeared that 1.27% of the pregnant women who were exposed to contagion, contracted the disease. If we compare this to the above figure of 3.7% in women of child-bearing age who were exposed and who were not protected, then it seems that this kind of prevention is quite effective, if the dose is sufficient (1,500 mg rather than just 750 mg) and if it is administered sufficiently soon after contagion.

However, this passive prevention is not always applied or it is applied too late, either due to negligence or to diagnostic difficulties (Young), because of the very discreet or even non-apparent forms. Even under the best application conditions, finally, it was found that the protection was not constant.

This is why the isolation of the measles virus -- which would enable us to tackle this prevention problem from a different and more effective angle -- has been most desirable for a long time.

Now, although the viral nature of the disease was no longer in doubt after the experiments involving transmission to human volunteers (Hiro, 1938; Anderson, 1949) or to monkeys (Hess, 1914; Habel, 1942), attempts at developing cultures nevertheless always failed.

But in 1962, 3 teams of North American virologists, working independently, isolated the German measles virus simultaneously, on cell cultures.

Weller and Neva managed to develop a culture from specimens of blood and urine taken from measles patients; they were able to grow these on human amniotic cells (in a primary culture). The virus multiplies only very slowly. The cytopathogenic effect appears only after several passages: first very slowly (17-24 days) and then rather discreetly, and after



that more rapidly and more definitely as the passages go on. The appearance of neutralizing antibodies from patients' serum proves the responsibility of the isolated agent in causing this disease.

Parkman and his associates used a different technique. They isolated the virus on cell cultures taken from monkey kidneys or human embryo kidneys. In spite of the absence of the cytopathogenic effect on these media, they were able to prove the fact of multiplication by an interference phenomenon with another virus (Echo 11). The latter, in effect, is no longer capable of multiplying and passing its usual cytopathogenic effect on to kidney cell cultures if they have been inoculated earlier with specimens taken from the throat of measles patients.

Antibodies which neutralize this interference phenomenon appear in the serum of patients.

Veronelli and associates obtained superposable or identical results on the basis of throat and blood samples [specimens], using an identical technique.

The identity of the virus isolated by these three teams of researchers was subsequently confirmed by serum-neutralization.

These studies were very quickly confirmed by other authors (Sever, Sigurdardottir, Balsamo, MacCarthy, Plotkin).

Certain other points have likewise been confirmed: the physical-chemical properties, particularly the resistance to cold and the sensitivity to ether; the impossibility of producing a complement deviation reaction, perhaps because of the weak concentrations in the viral particles that are obtained; absence of hemadsorption and hemagglutination; these culture viruses are successfully inoculated into volunteers (Sever), as well as into monkeys but there is no success here with the other laboratory animals.

Interference is found again in numerous other viruses (Sindbis, Coxsackie, polio, SV 4).

Certain findings are particularly interesting:

Among the various cell cultures sent, many permit the multiplication of viruses but a cytopathogenic effect is observed only in the human amniotic cells. MacCarthy recently showed that the same result could be obtained with a continuous-line culture of rabbit kidney, which is more practical to use.

In measles patients, the virus seems to be capable of isolation in more than 80% of the cases (Sever). The maximum success is obtained with 3 specimens from the throat (throat washing) during the first 3 days of the disease (Plotkin). Blood, urine, and even ganglion (MacCarthy) specimens can also be used.

An increase in neutralizing antibodies can be found in 90% of the cases. Neutralizing antibodies are found at significant rates in many children who have malformations of measles origin (Plotkin).

The isolation of the measles virus undoubtedly will have worthwhile applications in the future:

Virological diagnosis of the disease; serological diagnosis of the disease and discovery of receptive subjects; perhaps the perfection of an effective vaccination.

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### Vaccination Against Measles

An effective prevention of measles would not appear to be unjustified here. As a matter of fact, although child mortality due to this disease has decreased considerably over the past dozen or so years, particularly after the introduction of overinfection antibiotherapy, it nevertheless has not disappeared entirely. By way of example, we here in France, according to statistics prepared by the INSEE for the period of 1950-1960, had 3,136 deaths due to measles; during the same decade, we had 3,832 deaths due to whooping cough and 2,157 deaths due to poliomyelitis. According to Langmuir, mortality in the United States is supposed to be 1 out of 400,000.

In the underdeveloped countries, mortality is much higher. In the Indies, for example, Taneja found 1 death for every 300 children under the age of 5.

Measles thus remains a severe infection in tiny tots and in weak children. Certain superinfections [overinfections] are not always well treated or well controlled by antibiotics. Finally, the viral complications as such remain roughly what they were, although some of them do benefit from corticotherapy (pulmonary and laryngeal viral complications). The frequency of encephalitis in particular has not decreased. It is reported to be between 2 (Langmuir) and 1 out of 1,000 in the United States. The seriousness of this complication is well known. The mortality rate is not negligible and it can cause rather considerable psychic or neurological sequelae.

The possibility of preparing a vaccine came up when Enders and Peebles in 1954 demonstrated that the morbillous virus could be easily cultivated, accompanied by a cytopathogenic effect, on human and monkey kidney cell cultures.

Starting in 1958, many attempts and experiments were conducted mostly in the United States.

Two methods for preparing a vaccine from virulent material were used and compared; employment of a live but attenuated virus; employment of a strain of virulent but inactivated (that is to say, killed) virus.

#### Live Attenuated Vaccine

Much experimentation has been accomplished in the United States and Europe with attenuated live vaccine; it consists of a strain of virus (the Edmonston strain) whose virulence Enders was able to reduce by means of numerous passages over human kidney cell cultures and then over human amniotic cells, followed by passage over chicken embryo egg, and then finally on chicken embryo cell cultures. The moment the virus was adapted to the latter culture medium, it lost its capacity to produce experimental

measles in the receptive monkey although it retained the capacity of producing -- in that experimental animal -- an antibody titer comparable to that which appeared after this disease.

In other words, the virulence of the strain had been attenuated but its antigenicity has been preserved.

Right now, several tens of thousands of receptive children -- that is to say, children who have never had measles and who therefore do not have any anti-morbillous antibodies -- have been vaccinated with this attenuated Edmonston strain (as a matter of fact, two slightly different vaccines were used) in the United States, according to Enders, Katz, Lepow, Krugman, Karelitz, but also in Great Britain (Goffe) and in Finland (Halonen), etc.

From the first clinical experimentation it was learned that this live vaccine offered the advantage of providing good protection although it did entail the disadvantage of very often producing a strong general reaction, in other words, a veritable "little measles case."

The results, in a more detailed but schematic fashion, can be described as follows:

The effectiveness appears to be excellent.

As a matter of fact, the antibody titers (that is to say, neutralizing antibodies which deviated the complement or inhibited the hemagglutination), comparable to those observed after the disease, appeared in the serum of more than 95% of the subjects vaccinated, after just one subcutaneous injection.

They seem to persist for a long time at rather high titers, after vaccination; this seems to point to lasting protection. At any rate, going back one or two years, the authors have not found any measles in vaccinated subjects that were exposed to contagion.

There was no local reaction at the point of injection, but the general vaccination reactions unfortunately are frequent and very marked.

A fever reaction is manifested in about 80% of the cases (and sometimes even 100% of the cases with certain vaccine lots: Karelitz, Halonen). In general, it is around 39° C. But it can also turn out to be as much as 41° C.

This fever usually starts around the 7th day and lasts an average of 3 days.

An eruption occurs in 45-60% of the cases (85% of the cases in certain lots). This is an attenuated morbilliform eruption which is generally localized around the neck, the cheeks, and the upper portion of the trunk. It appears generally around the 10th day and lasts an average of 2 days.

A Koplick sign is noted rather often (10%-20% of the cases); here we can also observe a catarrh and coughing.

This vaccination reaction thus produces a kind of attenuated measles case.

Other complications however are possible: digestive troubles, otitis, amygdalitis, respiratory infections and even convulsions which nevertheless seem to be only hyperpyretic convulsions or the expression of a preexisting condition [comituality].

Indeed, no encephalitis accident has so far been reported but, in view of the low incidence of this complication, this negative aspect is not yet statistically significant.

However, as far as Gibbs is concerned, the EEG for children vaccinated with the attenuated strain of Enders never reveals the anomalies which we find sometimes in measles.

This "minor measles" case due to vaccination is not contagious; it is impossible to determine the presence of the virus in the throat or in the blood.

Nevertheless, it does constitute a handicap for the use of this vaccination.

Studies aimed at reducing the vaccination reactions and their complications by reducing the injected dose or by vaccinating only in the summer or in the autumn have not produced any significant results. As far as the method of introduction is concerned, we can only use the parenteral and nasal routes but the latter route seems to produce rather irregular results.

In other countries, vaccines involving other attenuated live strains according to the same principle as that used by Enders have been employed, (Zhdanov, Smorodintsev, in the USSR; Okuno in Japan). Results roughly identical to those summarized above were obtained.

#### Inactive Vaccines

An inactivated vaccine (killed) has already been experimented with, on a small scale, by Arakawa in Japan, with the help of a strain which he had managed to adapt, prior to the era of cell cultures, to the mouse brain and the embryo egg.

Then, Kempe in the US (1960) used a strain cultivated on monkey kidney and then inactivated; but these preliminary tests did not produce good results. As a matter of fact, immunization was not obtained regularly.

However, in view of the inconvenience due to the strong reaction from attenuated live vaccines, the inactivated vaccines were studied once again in 1961-1962 (Warren, Feldman, Karzon, Karelitz, Lipschutz, Frankel, Winkelstein, Carter).

Prepared on the basis of virulent strain, killed by various methods (ethylen oxide, formal), they turned out to be capable of producing good antigenic stimulation, provided they were administered in several injections given at intervals of 7 days to 1 month; an adjuvant was then added and the substance was above all concentrated so as to produce a high titer in the viral particles.

Carter, for example, obtained significant results with a vaccine that had been inactivated by formal, to which alumina had been added and which had been sufficiently concentrated likewise. The serums of 473 children (out of 5,000 vaccinated) were studied before and after injections. In 90%-100% of the receptive subjects, the antibodies appeared within 3-4 weeks, provided 3 injections were administered at intervals. However, the titers obtained (by sero-neutralization, inhibition of hemagglutination and deviation of the complement) are smaller than those observed after the disease or after live vaccine. They diminish rather rapidly and they disappear in about 6 months.

In already immunized subjects which have a certain antibody titer, the latter goes up rapidly after the injection or injections of killed vaccine (anamnestic reaction), contrary to what we observed in the case of live vaccine.

The local or general reactions were quite minimum.

Thus, it was possible to prove that an inactivated vaccine was sufficiently antigenic to produce an immunity response; however this response -- at least on the basis of the antibody titers -- is weaker and shorter than the response we get from attenuated live vaccine.

The promoters of this type of vaccination however, observed that the active immunity acquired here may well be sufficient because, if it does diminish, it might later on be strengthened through the anamnestic reaction during the period of incubation of a possible contamination and it might attenuate or prevent the appearance of disease symptoms.

In this connection we must point to the differences in terms of basic principle and the essential action between the live and the attenuated vaccines.

The inactivated vaccine is the only vaccine capable of bringing out a preexisting antibody titer (provided we are not dealing here with passively transmitted antibodies) by means of the anamnestic reaction, undoubtedly because of the good and immediate antigenic stimulation which it produces (when it is concentrated); but the immunity response, which it produces, seems to be shorter and less solid than the one we get from live vaccine.

The latter, on the other hand, is not capable of significantly bringing out a preexisting antibody titer, undoubtedly because this pre-existing immunity prevents it from developing or multiplying. Now, this is probably so because it multiplies, although very slightly because it is

capable of producing a good immunity response which is pretty close to that obtained in receptive subjects under natural disease conditions.

Overall, we can summarize the current state of experimentation on these two types of vaccines:

On the one hand, the attenuated live vaccine, which is administered in a single injection, enables us to obtain an antibody titer that is as high and durable as the one we get from the natural disease, as well as an immunity that appears to be quite solid although we do not have enough background data to judge this with certainty. But it does offer the considerable inconvenience of producing a frequent and rather important vaccination reaction, a veritable "minor measles case" which is not contagious.

On the other hand, the inactivated vaccine, is administered in 3 injections at intervals; it produces practically no general reaction but its antigenic stimulation is weaker; this gives us reason to think that the immunity conferred by it is not as good and not as durable.

In an attempt to avoid the inconveniences entailed in each of these two types of vaccines, 3 kinds of improvements have been proposed recently:

Association of gamma globulins with the attenuated live vaccine; successive utilization of killed vaccine and attenuated live vaccine; utilization of an over-attenuated strain of live virus.

For the purpose of diminishing the vaccination reactions after the use of attenuated vaccine, several authors have proposed that we associate human gamma globulins with them (MacCrumb, Reilly, Stokes, 1961). These gamma globulins are standard, commercially available human gamma globulins, in fact they are mixtures coming from a large number of serums, containing variable titers which however generally have enough neutralizing antimorbillous antibodies. They are injected right after the vaccine, in another place on the body.

The abovementioned authors, using this technique, achieved a significant decrease in the number of vaccination reactions (less than 10%-15%, according to the authors).

But there is also reason to fear a decrease in the immunization. Indeed, the titers obtained after this kind of vaccination are slightly weaker than those obtained with the live vaccine used alone. However, Stokes demonstrated that, a year after vaccination associated with gamma globulins, the neutralizing antibodies persisted as significant titers and that, parallel to this, a strong immunity was manifested because the children thus vaccinated were not in any way affected by measles epidemics. This particular author thinks that we can certainly hope for a long immunity in vaccinated individuals.

However, some objections might be raised to such a procedure:

The period of one year, which Stokes was working on, is insufficient to come up with a realistic judgement of the duration of immunity.

The neutralizing antibody titers vary from one gamma globulin lot to the other and are not absolutely standardized.

There is reason to fear that they might sometimes be insufficient.

However, the effective minimum dose seems rather weak: 12 (MacCrumb) to 20 (Stokes) neutralizing units per kilogram of weight.

Theoretically, we might also fear an inhibition of immunization if the dose is too strong. In very young infants, which still have neutralizing antibodies from the mother, the live virus does not vaccinate (Reilly).

However, the generally adopted doses -- 0.2 cc/kg -- do not seem to involve this inconvenience.

Here we have two injections instead of just one. The manufacturing cost is greater.

A second method consists in successively inoculating the inactivated vaccine and, one month later, injecting the attenuated live vaccine.

In 1963, Karelitz brought out the advantages of this method as part of a rather large-scale experiment.

The live vaccine very strongly brings out the neutralizing antibody titer which appeared after killed vaccine was inoculated in almost all cases (99.7%).

The killed vaccine to a great extent prevents the vaccination reactions of the live vaccine; this, by the way, depends on the number of prior inactivated-vaccine injections.

For a single injection of the latter (inactivated vaccine) (296 children tested), the live vaccine finally produces a fever reaction in 15% of the cases and an eruption in 3.7% of the cases.

When 2 injections are administered, we have 8% with fever and only 2 cases of eruption, out of 117 children tested. When we give 3 injections we have 1 case of fever and 1 eruption out of 75 children tested.

Thus it seems that this technique retains the advantages of the two vaccines while eliminating their inconveniences. The author proposes one injection of each of the vaccines at an interval of 1 month for children who can take this and 2 or 3 injections of inactivated vaccine, followed by 1 injection of live vaccine, in children that are weak.

This method, in this particular case, however does involve the inconvenience of requiring many injections.



A third method, in that diminishing the troublesome reactions due to the live vaccine while still retaining its excellent antigenic value, was proposed by Schwartz and Andelman (1962-1963).

It consists in the use of an over-attenuated (highly attenuated) live vaccine. This vaccine was prepared by means of 77 supplementary passages on chicken embryo cell cultures from the attenuated Edmonston strain.

This kind of vaccine was used (1 injection) by the abovementioned authors in 79 receptive children; they compared the results with a control group of 48 children who had been inoculated with a placebo.

The vaccination reactions turned out to be minimum: only 2.5% of those vaccinated had fever amounting to 39°C (2% for the placebo group). A discreet rash was observed in 11.3% of those vaccinated.

However, this vaccine seems to retain intact its antigenic power because 77 of the 79 vaccinated (97.5%) developed antibodies with high titers.

In another series of tests (without serological dosages), the authors used their highly attenuated vaccine on 475 children. None of these children were seriously inconvenienced. In 90% of them, the temperature remained normal. A slight eruption was reported in only 5% of the cases. No complications, particularly no neurological complications, were observed.

This highly attenuated vaccine thus appears to be a worth-while solution for the problem of antimorbilous vaccination because it seems to combine effectiveness and relative harmlessness quite nicely. However, further tests will have to confirm these preliminary data.

Generally speaking, all of the vaccines and vaccination methods, which we have just reviewed briefly, should be tested further on a larger scale and they should be compared. We can develop a meaningful opinion on their value only after this kind of large-scale testing effort after enough time has passed.

A number of unknowns still continue to exist, as a matter of fact, particularly on a subject of the possible neurotropism, something which we must always watch out for in dealing with attenuated live viruses, in spite of the reassuring preliminary data; other unanswered questions are connected with the subject of the duration and the strength of the immunity conferred, particularly by the inactivated vaccine.

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### Some Problems Created by Polio Vaccination

#### 1. Inactivated Vaccine or Live Attenuated Vaccine:

Polio vaccination has become possible since Enders, Wellers, and Robbins in 1949 showed that polio viruses multiply in cell cultures. This method, in effect, provided sufficient quantities of these three types of viruses (I, II, III) which were necessary to produce vaccines.

Two varieties of vaccines were proposed: inactivated vaccines and attenuated live vaccines.

These two are broadly used today, but the manner of employment varies from one country to the next. Let us briefly summarize the principles of production which differ greatly for these two vaccines.

The inactivated vaccines are concentrated and balanced suspensions of three types of inactivated poliomyelitis viruses, that is to say, viruses killed by one or more antiseptics (formol, beta-propiolactone). The virus particles thus lost their virulence and their ability to multiply while retaining their antigenic properties. They are administered in subcutaneous injections (Salk, Lepine vaccines).

Attenuated live vaccines are attenuated strains of virus I, II, and III, selected after many passages. They retain their ability to multiply in the vaccines, but they have lost their neurotropism. These vaccines are administered via the buccal route (Sabin, Koprowski, Cox vaccines).

We shall see that these basic principles must be slightly altered for one or the other vaccine varieties.

### What Are the Advantages and Disadvantages of these Two Vaccines?

Inactivated vaccines. The protection which they confer is very good, although it is neither constant nor fast nor perfect.

Although there is no strict parallelism between the idea of immunity and the idea of the presence of serum antibodies, the dosage of neutralizing antibodies nevertheless gives us an idea of the protection against each of the three types of viruses.

The results of these dosages varies slightly according to the origin of the inactivated vaccine (United States, France, Sweden) and even according to lots.

In the French inactivated vaccine, for example, the percentage of subjects immunized for the 3 types of viruses after 3 injections increased from 25%-30% (percentage of subjects naturally immunized by non-apparent infections) all the way up to 65%-75%.

But, if we consider only the triple-negative subjects (that is to say, those who are not naturally immunized against any of the three types) and if we look at them prior to vaccination, then we can see that the percentage obtained is much smaller. Now, this is precisely the case with children and it applies all the more, the younger they are. Thus, the percentage of vaccination immunization is correct -- after 3 injections -- only as of the age of 10.

Immunization is likewise variable according to the type of virus; it is better for Type I (which, by the way, is most wide-spread in France) than for the others.

The titers appear progressively but they do not reach their maximum until several weeks after the 3rd injection.

Then they diminish generally during the current year. But the follow-up injection, which is absolutely indispensable, and which must be performed 1 year later, brings the percentage of triple immunization up to a highly satisfactory figure of 90-97%, for all ages and for all 3 virus types (Lepine, Martin, Daguet).

Then, after a follow-up with the inactivated vaccine, the titers decrease slowly in a manner that varies according to the type of virus and vaccine (for the Salk vaccine, for example, the Type II antibodies stand up better than those of Type I and especially Type III). Subsequent follow-up injections, every 3-5 years, are advisable.

But it is especially the nature of immunity conferred by the inactivated vaccines that is very particular. Essentially humoral, it protects the nervous system and prevents paralysis but it does not prevent a non-apparent poliomyelitis infection, that is to say, the multiplication of wild poliomyelitis viruses in the intestinal cells and their spread in nature. The subject protected by the vaccine can thus, nevertheless, be a carrier of germs during an epidemic.

According to epidemiological surveys conducted in many countries, the inactivated vaccines cause a very considerable decrease in the incidence of paralytic poliomyelitis among the vaccinated population. But they do not eliminate it completely.

On the other hand, they do not prevent the circulation of wild viruses during epidemics among the population. They do not permit blocking an epidemic of poliomyelitis infection and they cannot lead to the eradication of this disease.

On the other hand, one of the major advantages of the inactivated vaccines is their harmlessness.

The accidents observed in 1955 in the US with the Salk vaccine (a case of poliomyelitis after vaccination) were in effect due to incomplete inactivation by the formol of the viral strains some of which were highly neurotropic. As a result of this it was found out that it was impossible to obtain complete inactivation by formol. Likewise, strains that had been stripped of their neurotropic power by the subcutaneous route were selected, as was done from the very beginning, by P. Lepine in the case of the French vaccine.

After that the harmlessness of the Salk vaccine was the same as that of the Lepine vaccine.

The tolerance is equally excellent if we keep in mind the major contraindications of any vaccination. In allergic individuals, however, we can have accidents. It is possible to prevent them by using the skin test and by means of certain precautions (Besredka method, antihistamines).

Parenteral administration is obviously less practical than oral administration of live vaccines but it is generally quite well accepted. It is possible to associate the inactivated vaccine with diphtheria and tetanus antitoxins and even anti-whooping cough vaccine, retaining a correct immunogenic power.

Overall, the inactivated vaccine -- which is quite remarkable because of its harmlessness -- most often guarantees good individual protection against neurological poliomyelitis, provided we administer the follow-up injections [booster shots] in the proper manner.

We can only hope for the wide-spread use of the inactivated Lepine vaccine in France which has produced excellent results over several years.

The attenuated live vaccines are being used increasingly in many countries.

The protection which they confer is faster and seems to be more constant than the protection derived from the inactivated vaccines.

Serological conversion obtained in a population after separate oral administration is about 95% for Types I and III. It is less frequent for Type II. Its duration seems to vary. But the effectiveness of live vaccines differs, in terms of its nature, from the effectiveness of inactivated vaccines. In effect, the immunity conferred is not only serological but also cellular, in other words, it is quite closely related to that of the natural disease. As in the non-apparent natural diseases, the virus vaccine multiplies in the intestinal cells and is excreted over several weeks. It can even contaminate the environment and this is generally considered an advantage ("immunity epidemics"). This guarantees intestinal cellular immunity and the vaccine thus avoids the natural infections due

to wild virus, in case of epidemics; virulent viruses cannot be excreted by the stool. The live vaccine is thus capable of blocking the expansion of an epidemic because this cell immunity seems to take hold very quickly.

On the other hand, the simultaneous vaccination of an entire population, made possible by the ease of oral administration and the lower production cost, seems quite capable of leading to the complete eradication of the disease, at least for a certain time, due to the arrest of the non-apparent circulation of wild viruses. The results reported in the various symposiums, particularly during the 9th European Symposium in Stockholm (September 1963) are very impressive. In the Eastern European countries (USSR, Czechoslovakia, Rumania, Hungary, Poland), hundreds of millions of subjects have been vaccinated with Sabin or Koprowski strains in the course of brief "mass" vaccination drives conducted simultaneously. This has produced a spectacular drop in poliomyelitis. In certain countries, the disease seems to have been eradicated completely. For example, Czechoslovakia did not have a single case of poliomyelitis in 1961, 1962, and 1963 (Skovranek). In many other countries, particularly the US and many of the Western European countries, live vaccines are also used very extensively with highly satisfactory results. Their mode of administration varies. The 3 types -- I, II, and III -- can be administered simultaneously or separately or they can be combined by twos at an interval of several weeks. The method of administration is always buccal. The vehicle used here varies (syrup, bonbons).

Nevertheless, the attenuated live vaccines do not seem to be entirely free of disadvantages, from the viewpoint of effectiveness and especially from the viewpoint of harmlessness.

From the viewpoint of effectiveness, there is reason to fear that an infection by enteric virus simultaneously with the vaccination, might through interference impair the multiplication of the attenuated virus. If we administer the 3 vaccination strains simultaneously, we might also have reason to fear that the immunization would be reduced, for one or the other, as a result of competition.

To avoid these inconveniences, whose incidence by the way seems to be rather minor, it is necessary to select the date for the vaccination drives on the basis of the local epidemiological conditions and the separate administration of the 3 strains would seem preferable.

The problem of harmlessness is much more important and is currently very much under study. During properly supervised vaccination drives, a number of cases of paralytic syndromes of the poliomyelitic type have been observed and reported in a number of publications. In certain countries (US and Canada) oral vaccinations were temporarily suspended.

Systematic investigations were conducted in several countries in order to spell out these facts. Considerable resources in terms of epidemiological, clinical, serological, and virological investigations were used. Here are the results:

A certain number of these paralysis cases, following virological and serological examination, turned out to be due to other viruses, that is to say, viruses other than the poliomyelitis virus (virus coxsackie, virus Echo). It is possible that this pathology might take on increased importance in the future, if the vaccination should reduce the sickness rate due to poliomyelitis.

In other cases, we were dealing with neurological attacks due to polio virus. But the problem was to determine whether the isolated strain was the vaccine strain or a wild strain. Various elements enable us to solve this problem to a certain extent: the identity of the antigenic type when the vaccination strains are administered separately; a study of certain different characteristics, for the wild virus and the attenuated virus, in other words, characteristics which are called "genetic markers" although the value of this method is rather disputed; arrangement of cases in terms of epidemic sources or random development.

In this way it was possible to determine that certain cases were due to wild strains whereas others were due to the vaccination strain.

The frequency of these manifestations is very low and varies according to the age and the type of virus. The reporting officials agree on these points in their different investigations.

We might mention here the investigation conducted in the US and reported recently by Gelfand (1963). This was a statistical project conducted by the contagious disease center of the US (Atlanta) in 1962.

The paralysis cases considered as "compatible" with the vaccinal etiology, after a critical and very severe analysis of all of the cases collected, numbered 18; this figure, compared to the number of subjects vaccinated in the US at the same time, gives us an idea of the "maximum potential risk" of such accidents: 1 out of every 4.4 million for Type I; 1 out of every 1.4 million for Type III.

No accident was reported for Type II.

On the other hand, "compatible" cases can be observed only among adults. Finally, the maximum potential risk (combined for Types I and III) is 1 out of every 500,000 for subjects of over 30 years of age. In fact, the real risk -- if we consider the fact that the cases reported are only "compatible" with the vaccination etiology -- is undoubtedly much lower as far as the author is concerned. After this investigation, the US government agencies concerned advised practitioners to use the oral vaccine for adults in full awareness of this small risk.

Other authors discovered that cortisone treatment causes an action that promotes physical fatigue and reduces general resistance.

Are these cases of vaccination paralysis due to a minimum persistence of neurotropism in the vaccination strains (particularly Type III)?

The non-apparent vaccination disease, in any case, at least in certain subjects, seems to be more important that we are inclined to think right now because post-vaccination viremias have been observed.

Are they due, on the other hand, to a recovery of the neurotropism after intestinal multiplication in the vaccinated individual and after dissemination into the environment? The virological studies conducted with the help of "genetic tracers" and experimental cerebral inoculation, before and after human passages, did reveal a certain instability on the part of the attenuated viruses (particularly III), but the results of these investigations were not very demonstrative. A number of virologists would appear to think that the possibility of a marked revival of the neuro-pathogenic power of the vaccination strains should be expected in the future. Others think that this risk is almost zero.

At any rate, the simultaneous oral vaccination of a population is quite in keeping with the desire to avoid successive passages of vaccination strains in man, and to stop the circulation of wild strains.

By and large, the attenuated live vaccines are widely used throughout the world and certainly have an undeniable effectiveness which, it seems, would make it possible to achieve the complete eradication of poliomyelitis -- if they are used in a massive and simultaneous fashion. But is the incident risk of vaccination paralysis acceptable in order to obtain this result?

The choice between the two vaccines (inactivated and attenuated) finally revolves around the criteria of harmlessness and effectiveness and is generally a function of the local sickness rate due to poliomyelitis and even the social structure of the people involved.

The use of two types of vaccines is furthermore possible in one and the same population.

## 2. Problem of Protecting the Newborn and Infants Against Polio

The protection of the newborn is very often guaranteed by antibodies transmitted from the mother.

Recently, Mayer and associates tried to figure out the frequency and duration of this passive immunity and to get an idea of its significance with respect to vaccination immunization in infants.

About 75% of the mothers had antibodies against the three types. An equal number of newborn was immunized by the passive transmission of these antibodies. The average half-life of these antibodies of maternal origin, calculated on the basis of repeated dosages in the infant, varies between 28 and 45 days. On the average, we find that a large proportion of infants is no longer protected as of the 3rd month. It thus seems desirable to vaccinate infants as of the 3rd month, if poliomyelitis is not to become a proportionally more frequent disease among infants than among the rest of the vaccinated population. When pregnant women are vaccinated during their pregnancy, the passive protection continues up to the



6th month. We must then wait for that age to be reached before we can perform a vaccination because a high antibody titer transmitted in this case may interfere with active immunization.

We can use the inactivated vaccine here. Booster shots are indispensable after the 3 injections.

The attenuated live vaccine can also be used in infants rather early (M. Lipson Lepow).

In pregnant women, it was used very extensively in all countries and no incidents were reported.

Recently, however, Just in Switzerland, found a rather high rate of abortion of still-born in pregnant women who were vaccinated during the first 3 months. This observation, the only one of its kind so far, requires confirmation and we must ask ourselves whether the vaccination strains might be at all responsible for this.

### 3. Problem of SV 40

We know that this latent monkey virus can infect a certain number of monkey kidney cell cultures which are used for growing polio viruses.

Originally, a large quantity of Salk vaccine contained this live virus because it is not inactivated by formol. Afterward it was demonstrated that this SV 40 was oncogenic for the newborn hamster.

On the basis of the theoretical fear that oncogenic properties might manifest themselves among vaccinated subjects, monkey kidney cell cultures containing this virus were then systematically eliminated from the production of these vaccines. The problem has thus been solved both for inactivated and for live vaccines.

We must also note that the French inactivated vaccine (Lepine) never contained SV 40 because of the use of non-infected monkeys (African monkeys). The antiseptic used here, moreover (Beta-propiolactone) would have been capable of inactivating this virus.

But in the US, many subjects were vaccinated many years ago with inactivated vaccines containing SV 40.

Fraumeni and associates used a statistical method and explored this population in order to determine whether the theoretical risk of oncogenic properties in man was confirmed. Fortunately, they did not find any significant increase in the cancer sickness rate among that population, with respect to the others and with respect to preceding years.

Other authors however noted that the quantities of SV 40 injected are very small.

Nevertheless, the period of time involved (the past 4 years) was deemed insufficient to form a definitive opinion.

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#### Curative Antiviral Therapy

In contrast to the tremendous progress that has been made in recent years in the field of preventing virus infections, we must mention the still very restricted range of curative therapy involved here.

The problems which must be solved in order to obtain active anti-virus medications, are highly complex.

The viruses are strictly cellular parasites. Let us briefly recall their mode of reproduction.

Viruses essentially consist of a central nucleic acid and a protein envelope.

They are fixed in an elective fashion on sensitive cells (through a specific receiver in the cell membrane).

The virus then penetrates the cell. It introduces an "information" (something like the genetic information) which is capable of diverting the

endocellular protein synthesis metabolism to the benefit of the synthesis of numerous virus particles (nucleic acids and protein envelopes).

The new viruses thus synthesized by the host cell are then liberated to the outside and can then contaminate the other cells.

Theoretically, it is thus possible to act upon the viruses during several stages of this cycle.

On the free viruses (exo-cellular); on the penetration into the host cell; on the endo-cellular viral synthesis.

Only this latter method facilitates the curative, it seems, and not just preventive therapy.

But the inhibitors of viral synthesis (which act in the manner of anticancer chemical-therapy agents), though they are effective, are also toxic for the cells of the infected organism.

This means that the products proposed -- products of a biological or synthetic origin -- currently involve two drawbacks: ineffectiveness and toxicity.

The most effective ones, because of their toxicity, can be used only locally (skin or mucous viroses). Those that can be used in the general fashion seem to be not sufficiently active.

One of us recently reviewed the various products and the limited therapeutical results which they have produced (Revue du Practicien, No 33, 21 Dec 1963) and we will not go into this again here.

The problem of the therapeutic applications of the interferon likewise has been taken up. We recall that this protein, which is developed by the infected cells by certain viruses, is capable of protecting the other as yet not infected cells.

We are thus dealing here with a natural cell defense against virus infection.

This substance is specific in various species. However, the interferon produced by monkey cells is capable of protecting human cells.

But the therapeutic effect is primarily preventive and its applications are thus extremely limited.